May 2004

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West Nile virus (WNV) has spread rapidly across North America, resulting in human deaths and in the deaths of untold numbers of birds, mammals, and reptiles. The virus has reached Central America and the Caribbean and may spread to Hawaii and South America. Although tens of thousands of birds have died, and studies of some bird species show local declines, few regionwide declines can be attributed to WNV. Predicting future impacts of WNV on wildlife, and pinpointing what drives epidemics, will require substantial additional research into host susceptibility, reservoir competency, and linkages between climate, mosquitoes, and disease. Such work will entail a collaborative effort between scientists in governmental research groups, in surveillance and control programs, and in nongovernmental organizations. West Nile virus was not the first, and it will not be the last, exotic disease to be introduced to the New World. Its spread in North America highlights the need to strengthen animal monitoring programs and to integrate them with research on disease ecology.

Keywords: West Nile virus, disease ecology, birds, mosquitoes, pest management

On 23 August 1999, the director of infectious diseases at the Flushing Medical Center in Queens notified the New York City Health Department that three admitted patients had an apparently neurological illness. The symptoms included fever, weakness, and confusion. As the number of similarly ill patients grew to five, doctors noted that many were elderly and had spent time outdoors on previous summer evenings. On the basis of this information, and the fact that one of the patients appeared to have encephalitis, the causative agent was suspected to be a mosquito-borne virus.

Meanwhile, American crows (*Corvus brachyrhynchos*) had been dying in large numbers in Queens, New York, since June. Unfortunately, this information was not reported to the Centers for Disease Control and Prevention (CDC) until 4 September, one day after St. Louis encephalitis (SLE) virus had been diagnosed as the causative agent in the human outbreak. Since birds infected with SLE are asymptomatic, public health officials viewed the crow die-off as unrelated to the cases of human illness.

At the Bronx Zoo, Tracey McNamara, a wildlife pathologist, had been conducting necropsies of crows since August. By September, a bald eagle (*Haliaeetus leucocephalus*), a snowy owl (*Nyctea scandiaca*), flamingos (*Phoenicopterus* spp.), cormorants (*Phalacrocorax* spp.), and other birds had unexpectedly died at the zoo. McNamara sent samples to the US Department of Agriculture's National Veterinary Services Laboratories (NVSL) in Ames, Iowa, suspecting that the birds might be ill with the same disease. NVSL workers discovered that the causative agent was a flavivirus, a family of viruses that includes both SLE and West Nile virus (WNV). This finding explained the CDC's positive test results for SLE in the human epidemic. At this point, NVSL contacted the CDC, because such viruses require Biosafety Level 3 containment facilities. Though skeptical of the NVSL findings, the CDC requested a tissue sample from McNamara on 19 September.

McNamara also contacted the US Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland. Both USAMRIID and the CDC confirmed that a flavivirus had killed the birds, and they started testing the samples against other flaviviruses. On 24 September 1999, USAMRIID and the CDC concluded that the birds had been infected with WNV (Steele et al. 2000). By the month's end, it seemed clear that humans and birds had died not from SLE but from WNV, a virus not previously detected in North America.

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As the end of the summer of 1999 approached, researchers and public health officials hoped that winter conditions would eradicate the infected mosquito population. Unfortunately, infected adult mosquitoes were able to overwinter in underground sewers, abandoned buildings, and bunkers (Nasci et al. 2001). These infected mosquitoes probably infected more birds the following spring, initiating a second transmission season and beginning the expansion of WNV throughout North America.

Since 1999, WNV has spread across almost all of North America, resulting in the deaths of more than 450 people and tens of thousands of birds, horses, and other animals (CDC 2003a). As of 4 September 2003, at least 208 species of birds, 29 species of native and exotic mammals, 1 species of lizard (*Varanus salvadorii*), and 1 species of alligator (*Alligator mississippiensis;* Miller et al. 2003) had exhibited WNV infection (USGS 2003). Seven Canadian provinces had confirmed cases of WNV in birds, equines, or humans as of October 2003. The virus was found in Jamaican resident birds by serology-based assays in 2002 (Dupuis et al. 2003) and in animals from the Dominican Republic (Komar O et al. 2003), Mexico (Estrada-Franco et al. 2003), and El Salvador (horses; Nart 2003) in 2003.

The short- and long-term impacts of WNV on wildlife are uncertain. The current and ultimate prevalence of the disease in mosquitoes, birds, humans, and other animals in the New World is also unclear. Elucidating these issues will require understanding the basic biology, coevolution, and environmental interactions of the virus, hosts, and vectors. Here we review current knowledge of WNV, its vectors and hosts, and its potential impacts on wildlife populations.

The virus and the vector

West Nile virus was first isolated in 1937 from a feverish woman in the West Nile district of Uganda (Smithburn et al. 1940). Since then, it has been isolated in western and central Asia, the Middle East, southern and eastern Europe, and the Western Hemisphere (CDC 2003a). Although its exact point of origin in the United States is unknown, it was probably introduced somewhere in New York during 1999. The US strain, now termed the NY99 strain, was genetically analyzed and found to be 99.8% similar to the strain isolated in 1998 from a goose (*Anser* sp.) that died in Israel (Lanciotti et al. 1999). West Nile virus is a member of the Japanese encephalitis antigenic complex (family Flaviviridae), a genus that is distributed worldwide (CDC 2003a). Arboviruses, including WNV, are transmitted among hosts by arthropod vectors (mostly mosquitoes and ticks). At least 80 of the known arboviruses cause human disease (Berge 1975). Most arboviruses depend on arthropods for transmission among their hosts, and thus temperate epidemics often occur in summer and fall, when mosquito and tick populations are at their highest levels. However, the southern United States and the tropics may experience almost year-round arboviral epidemics, because mild winters lessen arthropod die-offs and allow for winter mosquito activity.

Mosquitoes are thought to be the main WNV vectors. Currently, 43 mosquito species have been found positive for WNV (CDC 2003b). While some literature suggests that WNV infection has no effect on mosquitoes, other work shows that it can lower rates of mosquito survival (Goddard et al. 2002). Mosquitoes may live up to several weeks, and females may lay multiple batches of eggs over their life spans. The females of some species require vertebrate blood to produce each batch of eggs, and although blood from any animal would probably provide the required proteins, many species of mosquitoes feed preferentially on a specific source (e.g., birds, mammals, or reptiles) for their blood meal. The time between a mosquito's blood meal from an infected host and its subsequent infectious state varies by species and environment, but it can be as short as 4 to 5 days at high ambient temperatures (Dohm et al. 2002).

Mosquito species differ with regard to their competence (i.e., the effectiveness with which they can become infected, replicate the virus, and transmit it to subsequent hosts; Turell et al. 2000, 2001, 2003, Sardelis et al. 2001, Goddard et al. 2002). Among laboratory-tested species, infection rates vary, and different "doses" of WNV are required to cause infection in different species (Sardelis et al. 2001, Goddard et al. 2002). In addition, WNV does not reach the salivary glands in all mosquito species, so not all infected mosquitoes are competent as vectors or can equally transmit the virus to the vertebrate hosts they bite (Sardelis et al. 2001).

The roles of most of the known susceptible mosquito species in the WNV transmission cycle are currently unknown, but several of these species appear to be competent (Turell et al. 2000, 2001, Sardelis et al. 2001, Goddard et al. 2002). Species in the genus *Culex* appear to be the main vectors implicated in the avian amplification cycle of WNV, with *Culex pipiens pipiens* being important in the eastern United States, *Culex pipiens quinquefasciatus* in the South, and *Culex tarsalis* in the West (Bernard et al. 2001). A number of susceptible mosquitoes are opportunistic feeders that feed on both mammals and birds; these species include *Aedes albopictus, Aedes vexans, Ochlerotatus japonicus, Culex nigripalpus, Culex quinquefasciatus, Culex salinarius, Anopheles atropos, Anopheles crucians, Coquillettidia perturbans,* and *Deinocerites cancer* (Apperson et al. 2002). Several of these opportunistic species may be important bridge vectors that move the virus from the *Culex*–avian cycle to mammals. Clarifying the vector competency of these and other mosquito species is critical to understanding WNV's disease ecology in the Western Hemisphere.

Host species vary in their ability to infect mosquitoes. This is because hosts differ in their level of viremia, or the amount of a virus that replicates and circulates throughout their blood. Several vertebrate hosts, such as humans and horses, are considered reproductive dead ends for WNV because they do not typically accumulate virus particles at sufficient concentrations to infect mosquitoes (Bunning et al. 2002). Other vertebrate hosts may be inefficient reservoirs because their infective period is short, reducing the likelihood of a bit-

ing mosquito becoming infected. Yet in many bird species, high concentrations of the virus replicate and the birds remain infectious for long durations, leading to the potential infection of additional mosquitoes (Komar N et al. 2003).

Understanding the relationship between host viremia and the probability of infecting biting mosquitoes is extremely important and requires further study. Early research (Work et al. 1955) suggested that mosquitoes could be infected by feeding on hosts with virus concentrations as low as $10^{3.5}$ plaque-forming units (pfu) per milliliter in their blood. In contrast, some recent vector competence studies show very low infection probabilities from hosts whose viremia levels are below 10^5 pfu per milliliter (Turell et al. 2000, 2001, Sardelis et al. 2001). This difference is crucial, because many host species have virus concentrations between 10^3 and 10^5 pfu per milliliter in the days subsequent to infection (Komar N et al. 2003). Arthropod and vertebrate hosts may or may not exhibit symptoms of infection.

Basic mosquito behavior is also important to understanding the WNV transmission cycle. Such knowledge is complicated, as mosquito behavior and species assemblages vary spatially and temporally. For example, many mosquito species exhibit vertical height specialization for host seeking (Bellini et al. 1997, Bosak et al. 2001), and species may vary in their activity periods throughout the day, with some crepuscular (active at only dawn and dusk), others diurnal, and still others strictly nocturnal (Spielman and D'Antonio 2001). There may also be intraspecific geographic variation in WNV competency (Goddard et al. 2002). To model how WNV moves and persists, variation in mosquito behavior and ecology must be integrated with host dynamics, such as roosting, foraging, molting, and breeding behavior in birds. This will probably be most challenging in tropical regions, where mosquito diversity and life history are especially complex.

Ectoparasites such as ticks, louse flies, and fleas may also play a role in WNV's life cycle (Blackburn et al. 1990, Abbasy et al. 1993, Anderson et al. 2003). Laboratory studies with *Ixodes scapularis* failed to demonstrate transmission of WNV (Gregory D. Ebel, Arbovirus Laboratories, Wadsworth Center, Slingerlands, NY, personal communication, 2004), but further studies with other species are needed. The role of ectoparasites, if any, in amplification and transmission of WNV in North America is as yet unknown.

Birds as hosts of West Nile virus

WNV infection has been detected in at least 208 species of native and exotic bird species in North America (CDC 2003a). Researchers' understanding of how disease susceptibility and exposure interact across avian taxa is extremely poor. The data available to address species-specific susceptibility are primarily from surveillance of dead birds and are subject to multiple statistical sampling biases. Most surveillance has focused on sampling corvids (crows and jays), though other bird species are likely to be affected by WNV. Crows, which have been shown to be highly susceptible to WNV infection (Kramer and Bernard 2001, McLean et al. 2001, Komar N et al. 2003), are relatively large-bodied, tend to be abundant in suburban and urban areas, and are likely to be seen by the general public. Smaller species may be as just as susceptible, but their carcasses are less likely to be found and are often scavenged or deteriorate faster. The corvid focus of most state health departments makes interpretation of the available data difficult. Interpretation is further complicated because most counties stop testing dead birds after a predetermined number of confirmed cases of infection (mostly crows) are reported for an area.

Determining species-specific susceptibility is critical for understanding WNV ecology and for protecting threatened and endangered wildlife populations. Susceptibility to mortality from disease is related to factors including phylogenetic history (Rosenstreich 1980, Komar N et al. 2003), genetics, immunocompetency (Rosenstreich 1980, Zekarias et al. 2002), and behavioral ecology. To date, few experimental data exist on host competency. In the laboratory, blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*), house finches (*Carpodacus mexicanus*), American crows, and house sparrows (*Passer domesticus*) are the most competent reservoirs of 25 tested species (Komar N et al. 2003). Yet laboratory conditions do not reflect conditions in the real world. For example, data on WNV competency have not been evaluated with regard to variation in immunosuppression or corticosterone secretion (Ben-Nathan 1994) caused by captivity stress. Recent work on mosquitoes' feeding preferences suggests that crows and jays may not be common sources of blood for several WNV vectors (Apperson et al. 2002, Lee et al. 2002). Further research on the host susceptibility of selected avian and mammalian species should be a priority. This work should use reasonable sample sizes and allow time to follow the entire course of infection, measuring the length and titer of viremia to gauge host competency.

It remains to be seen whether birds can act as long-term reservoirs for WNV. If they can, then birds might provide another mechanism by which the virus could overwinter, possibly through virus recrudescence after the bird host completes its migration. Birds that survive infection probably maintain detectable viremia for only 1 to 7 days, after which the infection is cleared from the blood by the host's immune system (Komar N et al. 2003). Viral RNA persists in selected organs for longer (more than 4 months; Kristen Bernard, Arbovirus Laboratories, Wadsworth Center, Slingerlands, NY, personal communication, 2004), but it is not yet known whether a new transmission cycle could be initiated by a relapse to an infectious state.

Birds also become infected with WNV through means other than arthropod transmission. In a study by Nicholas Komar and colleagues (2003), ingestion of WNV in aqueous solution caused infection in house sparrows, common grackles, American crows, house finches, and great horned owls (*Bubo virginianus*). Similarly, ingestion of infected mosquitoes caused infection in house sparrows and mice (Komar N et al. 2003). Although these findings are preliminary because of the small sample size, the possibility of infection through

oral ingestion can have important implications for mosquito-feeding species, including swifts and swallows. It is not known whether the ingestion of infected prey has played a significant role in the mortality of wild birds. Vultures, raptors, corvids, and other species that feed on infected hosts or carrion may also be at risk. In addition to transmission through ingestion, contact transmission in the laboratory has been documented in four species: ring-billed gulls (*Larus delawarensis*), blue jays, black-billed magpies (*Pica hudsonia*), and American crows. These birds were in physical contact with infected cage mates and may have become infected through oral–fecal transmission or allopreening (McLean et al. 2001, Komar N et al. 2002, 2003).

Nestlings may also be important virus reservoirs. Their feeding requires beak-to-beak contact with adult birds, which may facilitate horizontal transmission, as may the close proximity of nestlings to each other. The limited mobility of nestlings and their exposed, unfeathered skin presumably make them easy prey for mosquito vectors. Four of five blue jay nestlings submitted for testing in Ohio tested positive for WNV (Garvin et al. forthcoming). Also, three dead peregrine falcon (*Falco peregrinus*) nestlings from one nest tested positive in 2003. The fourth nestling from the same nest later tested positive for WNV, and the adult male was found to be antibody positive. In addition, because a large percentage of passerine songbird nests are depredated (Stephens et al. 2004), transmission to predators may take place through the ingestion of infected nestlings.

Nonvector modes of transmission could also increase infection risk in social species, such as crows. American crows are cooperative breeders that live in extended family groups consisting of a breeding pair and a variable number of auxiliaries, many of which are offspring from previous years (Verbeek and Caffrey 2002). Crows may attend and feed sick or injured group members (Kevin McGowan, Cornell Laboratory of Ornithology, Ithaca, NY, personal communication, 2004) and frequently allopreen (Verbeek and Caffrey 2002). Both behaviors potentially put crows in contact with viral particles in secretions and on the skin (Komar N et al. 2003). These behaviors may also spread ectoparasites from infected individuals to the uninfected. Crows roost communally, and although their giant winter roosts (some can have more than two million crows; Verbeek and Caffrey 2002) are unlikely to play a part in WNV transmission (Chu et al. 2003), summer and autumn roosts may be contributing to the spread of WNV in American crows (Anne B. Clark and Douglas A. Robinson, Department of Biological Sciences, Binghamton University, Binghamton, NY, and Kevin McGowan, Cornell Laboratory of Ornithology, Ithaca, NY, personal communications, 2004).

Flocking social birds, including waterbirds, may also be at high risk of WNV infection. Work and colleagues (1955) showed that buff-backed herons (*Ardeola ibis*) in Egypt were commonly infected (68% prevalence) and capable of transmitting WNV to mosquitoes. Malkinson and colleagues (2002) documented lethal WNV infections in white-eyed gulls (*Larus leucophthalmus*), domestic geese (*Anser anser domesticus*), and migrating white storks (*Ciconia ciconia*) in Israel. Hubálek and Halouzka (1996) observed mortality in inoculated black-tailed gulls (*Larus crassirostris*) in Russia. In North America, several large mortality events involving ring-billed gulls and American white pelicans (*Pelecanus erythrorhynchos*) occurred in 2002 and 2003; these events are thought to be due to lethal WNV infections. In contrast, 12 mallards (*Anas platyrhynchos*) inoculated with WNV, as well as 6 non-inoculated ducks with which they shared a common pool of water, showed no signs of clinical illness, and necropsies conducted 7 days after inoculation did not show virus present in any of their tissues (Douglas Docherty and Louis Sileo, National Wildlife Health Center, US Geological Survey, Madison, WI, personal communication, 2004).

Other vertebrate hosts

West Nile virus is unusual among flaviviruses, at least in North America, because of the large range of hosts it can infect. The CDC has reported infections of the virus in 29 species of mammals, including eastern chipmunks (*Tamias striatus*), striped skunks (*Mephitis mephitis*), fox squirrels (*Sciurus niger*), gray squirrels (*Sciurus carolinensis*), gray wolves (*Canis lupus*), sheep (*Ovis domesticus*), Rocky Mountain goats (*Oreamnos americanus*), big brown bats (*Eptesicus fuscus*), harbor seals (*Oryctolagus cuniculus*), domestic cats (*Felis domesticus*), and dogs (*Canis familiaris*). American alligators have also been infected; one farm in Georgia lost more than 1000 animals in 2001 and 2002 (Miller et al. 2003). Thus far, no dead wild alligators have been found with WNV infection, but this could be due to a lack of monitoring.

Other vertebrates may also play a role in WNV amplification. In Russia, WNV was isolated from frogs (*Rana ridibunda*) that were subsequently shown to be competent hosts for infecting *Cu. pipiens* mosquitoes. In North America, a Texas tortoise (*Gopherus berlandieri*) was shown to maintain high concentrations of an alphavirus, western equine encephalitis (WEE), for 105 days (Bowen 1977). The mosquito *Uranotaenia sapphirina* has been shown to be a competent WNV vector (Kostyukov et al. 1986), and in North America it bites both amphibians and reptiles (Cupp et al. 2003). Recent research has shown that garter snakes (*Thamnophis sirtalis*), red-eared sliders (*Trachemys scripta*), green iguanas (*Iguana iguana*), and bullfrogs (*Rana catesbeiana*) are all incompetent reservoirs (Klenk and Komar 2003). Further research on North American reptiles and amphibians is necessary to determine the importance of these animals in the WNV life cycle.

The physiological impact of West Nile virus on hosts

West Nile virus is neurotropic and tends to concentrate in brain and nerve cells, causing inflammation of these tissues (encephalitis and meningitis), fever, local bleeding, and cell death. In birds, initial symptoms of disease include anorexia, weight loss, sleeping, and pinching off of blood feathers. Birds in more advanced stages experience head tremors,

Figure 1. Symptoms of West Nile virus may include (a) head tilt (great horned owl), (b) vision problems (red-tailed hawk), and (c, d) lethargic and catatonic states (red-tailed hawk and bald eagle). Photographs: Marge Gibson.

green urates (indicating liver necrosis), central blindness, unawareness of surroundings, clumsiness and weakness in the legs, and more severe tremors and seizures just before death (figure 1; Patrick T. Redig, College of Veterinary Medicine, University of Minnesota, personal communication, 2004).

Infection survivors may suffer brain damage or other deleterious physiological effects (Petersen and Marfin 2002). Data on surviving wild birds are not available, but malfunctioning brains and bodies could potentially have profound effects, including interference with navigational skills. The internal pathology reported in surviving birds from some species includes damage to the kidneys, pancreas, and heart (figure 2; Steele et al. 2000). Survivors that appear asymptomatic after WNV infection are not necessarily unharmed. Ten sandhill cranes (*Grus canadensis*) that were infected experimentally exhibited no clinical illness 41 days after infection, even though necropsies conducted the same day showed histopathological lesions caused by encephalitis, myelitis, and myocarditis (Louis Sileo, Douglas Docherty, and Kimberli Miller, National Wildlife Center, US Geological Survey, Madison, WI, and Glenn Olsen, Patuxent Wildlife Research Center, US Geological Survey, Laurel, MD, personal communications, 2004).

The infectious state of WNV survivors is eventually terminated by their immune systems. After viremia wanes, virus particles and viral RNA can still be found in the tissues of some birds (Komar N et al. 2003; Kristen Bernard, Arbovirus Laboratories, Wadsworth Center, Slingerlands, NY, personal communication, 2004). The skin of one killdeer (*Charadrius vociferus*) contained 100,000 virus particles per milliliter 13 days after the virus cleared from its blood. Particles of WNV have been detected in birds' ovarian and testicular tissues (Komar N et al. 2003), raising the possibility that adult birds may pass the virus to their eggs. In addition, high virus concentrations have been seen in some bird kidneys (Kramer and Bernard 2001, Komar N et al. 2003), and the majority of the 24 bird species tested had virus particles in their oral cavities and cloacae during infection. These findings may have important implications for fecal–oral transmission. Furthermore, American crows, common grackles, and blue jays showed elevated levels of virus particles in their blood, on oral swabs, and in their cloacae through day 5 (Komar N et al. 2003).

The duration and variability of immunity among animals surviving WNV infection is essentially unknown. This is a problem for researchers who use serum antibodies as a sign of previous infection. Although positive serological results from an enzyme-linked immunosorbent assay (ELISA) suggest that a bird has been exposed to WNV, the results generally do not indicate when the infection occurred.

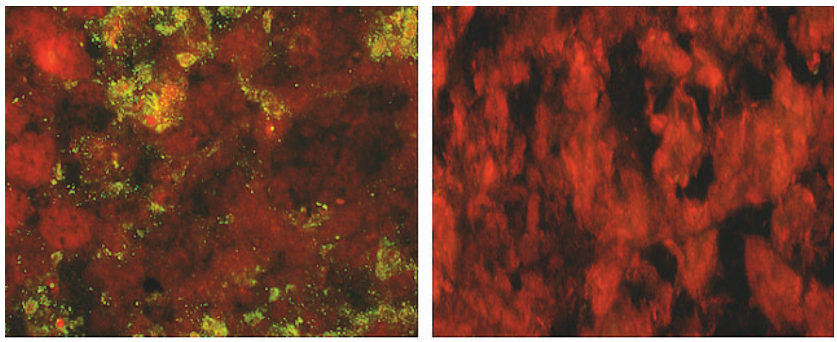

Figure 2. American crow kidney tissue sections (5 micrometers thick, magnified 200 times under ultraviolet light). The image at right is a normal kidney. The image at left is a kidney infected with West Nile virus (WNV). The WNV antigen is shown in green. Photographs: Arbovirus Laboratories, New York State Department of Health, Wadsworth Center.

Population impacts of West Nile virus

Researchers know that WNV has killed tens of thousands of birds and suspect that it has killed 10 to 100 times that many. It is difficult to measure the lasting impact of disease on wildlife populations, in part because defining a population geographically is complex, especially for migratory birds. Studies of marked populations, especially studies using radio transmitters, provide the best estimates of population impacts, yet few data from such studies have been published.

To date, the best information available is for American crows and greater sage-grouse, both of which appear to be heavily affected by WNV. Almost 100% of the American crows infected with WNV under laboratory conditions have died (McLean et al. 2001, Komar N et al. 2003). The virus first hit a marked crow population in Ithaca, New York, in 2002, with a mortality rate of about 33% in one area inhabited by 10 family groups (approximately 68 birds). Twenty-five birds disappeared from the population; of these, 21 were found dead. In 2003, a set of 23 families (approximately 168 birds) experienced a loss of at least 35% (Anne B. Clark and Douglas Robinson, Department of Biological Sciences, Binghamton University, Binghamton, NY, and Kevin McGowan, Cornell Laboratory of Ornithology, Ithaca, NY, personal communications, 2004). Also, one of the authors (C. C.) found that approximately 33% of 120 marked crows in Stillwater, Oklahoma, died within two months of WNV's arrival in September 2002. Nineteen of 28 crows tracked with radio transmitters (68%) died from June through October 2002 in Champaign-Urbana, Ilinois; all of these crows tested positive for WNV (Yaremych et al. 2004). Preliminary data using radio transmitters on greater sage-grouse during the breeding season of 2003 in Wyoming and Montana indicate that the grouse are vulnerable to WNV infection, but mortality rates vary greatly across the landscape (Brett Walker, College of Forestry and Conservation, University of Montana, personal communication, 2004).

The impact of WNV on populations of other bird species and over larger geographic regions is just now being studied. There are two primary data sets available for quantifying the impact of WNV over large areas: the Christmas Bird Count (CBC; *www.audubon.org/bird/cbc/*) and the North American Breeding Bird Survey (BBS; *www.mp2-pwrc.usgs.gov/bbs/*). Recent analyses using these data sets have revealed mixed results (Bonter and Hochachka 2003, Caffrey and Peterson 2003; John Sauer, Patuxent Wildlife Research Center, US Geological Survey, Laurel, MD, personal communication, 2003). In an analysis of CBC data, American crows and great horned owls showed weak regionwide declines (Caffrey and Peterson 2003), whereas most other species in the CBC and BBS counts showed only local declines. This suggests that common species are not at risk of extinction. Such mortality patterns may allow for regional recovery by immigration from unaffected areas, if populations are able to evolve disease resistance.

Nonetheless, a number of threatened and endangered species in the United States (including Hawaii), Central America, South America, and the Caribbean may be at serious risk of extinction because of WNV. Several species are already at low population levels because of habitat destruction and loss, introduced predators and competitors, diseases, and other anthropogenic stressors. Hawaiian birds are particularly vulnerable and are already in peril because of introduced diseases, including avian malaria (*Plasmodium relictum;* Van Riper C et al. 1986) and avian pox. Long-distance migrant birds such as the pacific golden plover (*Pluvialis fulva*) could bring WNV to the Hawaiian Islands, or humans could import infected birds. In response to the threat posed by imported birds, the US Postal Service and the Hawaiian Department of Agriculture have put in place a postal embargo and state quarantine for all birds carried into the state. All legally imported birds must be quarantined for a minimum of 7 days, unless they come from currently WNV-free states. Airplanes, passenger ships, and container vessels transporting infected reservoir hosts or vectors are another possible route of virus introduction into Hawaii.

Hawaiian birds are likely to be immunologically naive to arboviruses such as WNV, because they evolved in the absence of biting insects (Van Riper C et al. 1986). As yet, there is no evidence that any of the mainland North American arboviruses are present in Hawaii, including the WEE, SLE, and eastern equine encephalitis (EEE) viruses (Van Riper SG and Van Riper C 1985). However, there are a number of introduced birds (e.g., house sparrows and house finches) and mosquitoes (e.g., *Cu. quinquefasciatus*) that could support WNV amplification in Hawaii and transport it from low to middle and high elevations where remaining endemic passerines survive. This suggests that native Hawaiian birds, such as the remaining 37 Hawaiian crows (*Corvus hawaiiensis*) in captivity, could be highly vulnerable to WNV.

Movement and prevalence of the virus

West Nile virus rapidly spread across North America from the New York region after 1999. Multiple dispersal agents were probably involved in the movement of the virus, including infectious migratory and resident birds, dispersing mosquitoes, and human-assisted mosquito movement (e.g., mosquitoes moved through cargo containers such as trains, trucks, and airplanes; Lusina et al. 2000). Many mosquitoes do not move far from their hatch sites on their own, although individuals of some species are known to move up to 0.89 kilometers per day (Tietze et al. 2003). Viremic birds are currently presumed to be the main dispersal agents of WNV.

Though avian migration primarily flows north and south, east–west migrations occur in some species, and mixing of populations at staging and wintering areas is common. In addition, postfledgling dispersal and the normal wandering of individuals outside the breeding season have probably contributed to moving the virus westward. Long-distance migratory birds are not necessary to explain the spread of WNV over large distances; the virus could have been moved shorter distances sequentially by multiple individuals of short- and long-distance migratory species (Rappole and Hubálek 2003). Several WNC outbreaks have been linked to migrating birds in Europe (Zeller and Murgue 2001, Malkinson et al. 2002). Long- and short-distance migratory birds have been linked to the spread of similar viruses in the past. For example, evidence suggests that the 1962 EEE epidemic in Jamaica resulted from migratory birds carrying the virus from the continental United States (Work and Lord 1972). Yet conclusive data implicating viremic birds as major dispersal agents of WNV in North America are currently not available.

Given the size and varied landscape of North America, WNV propagates and amplifies through a number of different species assemblages (vector and host) and under a variety of environmental conditions. The distribution of competent vectors shares underlying factors with the virus's distribution. For example, the distribution of mosquito breeding sites depends on suitable habitat and on local microclimatic conditions, including temperature, humidity, precipitation, wind patterns, incident radiation, and seasonal weather patterns (Monath 1980). Landscape features such as topography, soil type, soil moisture, surface water, salinity, and general water quality may also play important roles in the reproductive success of competent vector species. Finally, geographic distance to other infected areas may influence local WNV prevalence and rate of spread.

Epidemics of WNV may be preceded by a particular series of climatic events (Epstein and Defillipo 2001). Such events illustrate that abiotic conditions influence where and when WNV flourishes in vector and reservoir hosts. For example, mild winters may enhance mosquito survival. In years when such conditions are followed by a dry spring and summer, birds may congregate around dwindling water sources where mosquitoes breed, thus increasing their probability of infection. At the same time, a drought can kill off mosquito predators (e.g., lacewings, ladybugs, dragonflies, and frogs) and concentrate rich organic materials in stagnant water, further optimizing breeding conditions for *Culex, Aedes,* and other mosquito species.

Wet springs and hot, dry summers may also facilitate WNV epidemics. Such was the case in the New York City area in 1999 and in Colorado in 2003, when the production of mosquitoes in higher than normal numbers was followed by maximized rates of viral replication within mosquitoes (because of warm temperatures; Dohm et al. 2002), leading to shortened transmission and amplification cycles. Torrential rains at summer's end can also lead to heightened amplification by causing an increase in ovipositioning sites. Given these scenarios, the increase in extreme weather events predicted by the Intergovernmental Panel on Climate Change may lead to future WNV epidemics as bad as, if not worse than, those in 2002 and 2003.

Local land-use patterns can also affect vector population dynamics and vector–host interactions. Water management strategies can influence where and when suitable vector breeding sites develop by influencing the size of wetland areas. In addition, urban and suburban settings provide numerous oviposition sites, such as gutters and discarded tires, for several container-breeding vector species. Sewer systems are known to provide refuge for infected mosquitoes over the winter (Nasci et al. 2001).

Management of an invasive disease

In 2003, Fort Dodge Animal Health developed a WNV horse vaccine known as West Nile–Innovator. Birds inoculated with this killed WNV vaccine, or with vaccines made from a similar virus, have displayed varying resistance; some species appear to acquire protection, while others do not. Sandhill cranes vaccinated with West Nile–Innovator stopped shedding viral particles from 0 to 7 days after WNV challenge, whereas unvaccinated conspecifics were still shedding up to 11 days later. Vaccinated individuals had internal lesions of lower severity than unvaccinated individuals upon necropsy (Glenn Olsen and Kimberli Miller, Patuxent Wildlife Research Center, US Geological Survey, Laurel, MD; Louis Sileo and Douglas Docherty, Wildlife Health Center, US Geological Survey, Madison, WI; personal communications, 2004). Forty percent of American crows inoculated either with Fort Dodge's West Nile–Innovator vaccine or with Japanese encephalitis vaccine survived challenge with WNV, compared with none of the controls. Recently, a DNA vaccine—a "living" mimic of WNV's RNA—was tested on fish crows. Those that were injected intramuscularly were completely protected from infection, whereas those that received oral doses did no better than unvaccinated individuals (50% survived subsequent challenge with WNV; Turell et al. 2001).

Effectively distributing WNV vaccines to wildlife populations will be problematic, if not inappropriate; large-scale vaccination could prevent the evolution of WNV resistance in natural populations. However, development of effective vaccines could offer a reprieve from WNV's lethal effects for threatened, endangered, and captively bred species.

Mosquito control is one of the more popular methods for controlling zoonotic diseases, or animal diseases that are naturally communicable to humans. Unfortunately, control measures are not always effective, because some of the entomological data needed to manage emergencies are lacking (Novak and Lampman 2001). Adult mosquito control may be applied at inappropriate times of the day or too late in the season to respond to human WNV outbreaks (Novak and Lampman 2001). The most effective approaches apply larval control measures to known problem areas in April or May. However, adult control at this time is problematic, because the densities of adult mosquitoes are low. Adult mosquito control is best applied when densities are at or slightly below their peak (Novak and Lampman 2001). This may help decrease the peak prevalence of WNV in birds, since peak mosquito densities lead to increased amplification of WNV. Defining high-risk areas for WNV will require the compilation of information on vector abundance and prevalence in near-real time.

Improved integration of mosquito surveillance and animal monitoring programs will greatly facilitate researchers' understanding of WNV outbreaks. Integrated pest management (IPM), which seeks to achieve favorable economic, ecological, and sociological consequences, may be the best available solution to control the virus while minimizing secondary impacts on wildlife. In the past, IPM focused on limiting negative effects on humans, but the recent concern over species threatened or endangered by WNV has led to the incorporation of wildlife protection measures. Using a three-pronged approach, IPM controls vectors where they are most concentrated, immobile, and accessible (Novak and Lampman 2001). For mosquitoes, this occurs when mosquitoes are still at the egg stage. For example, on one 16-hectare Illinois floodplain plot, a 15-centimeter square contained 367,000 eggs (James L. Regens, Civil Engineering and Environmental Science, University of Oklahoma, personal communication, 2004). But only 1 percent of the plot actually produced mosquitoes. By concentrating on such "hotspots," control measures may be effective. Unfortunately, control measures will be difficult to apply to some mosquito species, such as those in the *Culex* genus, because they tend to oviposit in localities that are dispersed and hard to reach.

Modeling may yield insight into WNV's distribution and prevalence across space and time and thus inform management strategies (Regens and Hodges 2000, Gu et al. 2003). Adaptive management uses multiple management models to reflect uncertainty about system response to management actions. Initially, all management models may be weighted equally to develop an optimal management action through optimal stochastic control methods. Using a Bayesian updating procedure, the natural system's response is then compared with the predictions of each model, and the models that best predicted the system's response are given more weight. Over time, the repeated confrontation of model predictions with reality should accumulate weight for the most accurate model, strengthening the predictive power of the model set and the effectiveness of management (James D. Nichols, Patuxent Wildlife Research Center, US Geological Survey, Laurel, Maryland, personal communication, 2004).

For adaptive management to mitigate the next WNV epidemic, monitoring feedback will be required. Two possible scales of monitoring may be useful, depending on the available management actions and their predicted effects. At the population level, three possible disease states of organisms (susceptible, infected, and recovered) need to be estimated. The survival rates in these states, and the transition rates between them, are the models' vital rates. At a larger scale, an estimate is needed of the proportion of areas with WNV-infected animals. The models' vital rates in this case are disease extinction and colonization probabilities for monitored areas, and the model state variables (proportions of areas with the disease present) are estimated on the basis of sampled animals. Accurate estimation of these state variables and associated vital rates is paramount for proper implementation of adaptive management.

A problem with any modeling approach is that models are only as accurate as their least accurate data. For example, the number of dead birds collected in an area is a function of human density (Theophilides et al. 2003). To control for such biases, records of dead bird surveillance should quantify sampling effort. The detection probabilities of WNV should also be incorporated into adaptive management models.

Research priorities for understanding West Nile virus

To understand the spread, distribution, prevalence, and ultimate impact of WNV on wildlife populations, a standardized system for reporting dead bird surveillance data across North America is required. Surveillance data must include monitoring effort as well as total sample sizes of dead birds reported and collected, including those that test negative. Unfortunately, most states cannot afford to support detailed surveillance or diagnostics, and there is a significant shortage of diagnostic laboratories capable of processing and testing specimens. One alternative to laboratory testing of carcasses would be a reliable WNV virus blood test that is accurate and inexpensive and could easily be used in the field. The recently available VecTest, in which swabs from oral or cloacal cavities are immediately assayed for the presence of WNV antigens, has worked with some success with mosquitoes and dead crows (Lindsay et al. 2003, Yaremych et al. 2004), but the number of false positives weakens the test's usefulness for diagnostic purposes.

Understanding how WNV moves across the landscape will facilitate researchers' ability to predict where and when outbreaks may occur. Identifying organisms that are best suited to serve as early warning sentinels will enable the targeting of surveillance and remedial efforts. Mosquitoes have not proved useful in this regard, and sentinel chickens' utility has varied geographically. The appearance of WNV in dead birds and horses appears to provide the earliest and best warning system in most regions of the United States (Guptill et al. 2003, Mostashari et al. 2003). Equally important in predicting and understanding outbreaks is detailed laboratory information on the variability of host species to in-

fection, including initial viremia, reservoir competency, antibody response, viremia longevity, and relapse potential.

Managing land to reduce the reproduction of arbovirus vectors could offer many advantages, but management may be complicated by policymakers' perception of conflict between public health, vector control, and wildlife preservation. Potentially infective mosquito populations can be managed by developing targeted control protocols and by altering how people manage, create, and reclaim wetlands. Broadscale pesticide treatment of wetlands and other natural areas may harm humans and nontarget organisms. By acting wisely regarding the reclamation of wetlands, the protection of natural areas, and the creation of protocols for land use and development, managers can minimize suitable habitats for virus-carrying mosquitoes (Metcalf 1998, Novak and Lampman 2001).

The spread of WNV in North America teaches scientists several humbling lessons regarding our ability to protect wildlife populations, especially threatened and endangered species, against invasive diseases. It also underscores how much more there is to learn about disease ecology. Researchers need to integrate their disciplines to develop effective strategies for disease surveillance and monitoring. Collaborative projects that combine basic research and applied management will be particularly important, especially in the face of continued shortages of research funds. Avenues are required for the communication and sharing of data between scientists and the surveillance and management communities. By establishing an organized response strategy to an invasive disease like WNV, scientists and managers will be in a better position to assess the risk posed by the next exotic pathogen introduced to North America and to limit its damage.

Acknowledgments

On 5–7 February 2003, a workshop entitled "West Nile Virus and Wildlife Health" took place at the Smithsonian Environmental Research Center in Edgewater, Maryland. It was convened to coordinate research and management priorities with the ultimate goal of understanding the impacts of West Nile virus on wildlife populations. More than 80 scientists listened to 30 speakers and participated in strategy discussions during the 2-day meeting. This article owes much to their commitment and to their storehouse of knowledge. Partial funding was provided by NIAID–NIH contract no. NO1-AI-25490 to L. K. The Smithsonian Institution's Office of the Undersecretary, the National Audubon Society, the US Department of Agriculture, the US Geological Survey, and the Centers for Disease Control and Prevention provided financial and logistical support for the meeting.

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